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IMMUNOTHERAPY FOR PROSTATE CANCER USING RECOMBINANT BACILLE CALMETTE-GUERIN EXPRESSING PROSTATE SPECIFIC ANTIGENS

Abstract:

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(54) Title: IMMUNOTHERAPY FOR PROSTATE CANCER USING RECOMBINANT BACILLE CALMETTE-GUERIN EXPRESSING PROSTATE SPECIFIC ANTIGENS

(57) **Abstract:** The present invention relates to the treatment of prostate cancer. Methods and compositions comprising recombinant BCG are provided for eliciting potent immune responses against prostate specific antigens that are effective for treatment of prostate cancer and metastatic disease.

IMMUNOTHERAPY FOR PROSTATE CANCER USING RECOMBINANT BACILLE CALMETTE-GUERIN EXPRESSING PROSTATE SPECIFIC ANTIGENS

FIELD OF THE INVENTION

[0001] The present invention relates to the treatment of prostate cancer. Methods and compositions comprising recombinant BCG are provided for eliciting potent immune responses against prostate specific antigens that are effective for treatment of prostate cancer and metastatic disease.

BACKGROUND OF THE INVENTION

[0002] Prostate cancer (CaP) is now the most common cancer in American men, with approximately 180,400 new cases estimated for the year 2000. In 1990, CaP surpassed lung cancer as the most commonly diagnosed cancer among American men. Approximately 189,000 cases, or thirty percent of all newly diagnosed cancers in American men in 2002 will be CaP. One in six American men will be diagnosed with CaP in his lifetime, and this cancer is the second leading cause of cancer deaths in American men with approximately 30,200 deaths estimated for the year 2002.

[0003] Ninety percent of CaP cases where the cancer is confined to the prostate (i.e., "organ-confined") can be cured with surgery if discovered early, but because there is no effective systemic therapy for this disease, the prognosis is poor once the tumor has spread beyond the gland itself and about half of the patients with CaP have clinically advanced (i.e., extraprostatic/extracapsular) disease at the time of initial diagnosis. Even in those patients initially determined to have organ-confined disease, one-third actually have undetected micrometastatic disease, as determined by subsequent pathological staging or disease progression. In all, more than 65% of patients with CaP develop metastatic disease.

[0004] Immunotherapy for the treatment of metastatic prostate cancer is based on the activation of the host's immune response against tumor-associated antigens (TAA) present on tumor cells that distinguish them from normal cells. TAA may be normal, tissue-specific cellular proteins that are upregulated on cancer cells, mutated

proteins, oncofetal antigens, growth factor receptors, oncogene and tumor-suppressor gene products, among others.

[0005] Prostate cancer is an ideal candidate for immunotherapy for many reasons. There is a substantial failure rate of current therapies for the primary tumor and a lack of effective chemotherapy for metastatic disease. The prostate contains organ-specific TAA that can serve as targets of an immune response. Because the prostate is not essential, its removal or destruction in many patients with CaP eliminates the concern for potential autoimmune disease. Moreover, immunotherapies can be directed at metastases without concern regarding the tissue of origin.

[0006] One immunotherapeutic approach for cancer involves the use of a patient's tumor cells mixed with various adjuvants, including cytokines, or genetically modified autologous cells that secrete cytokines. Hwang et al., *Semin Oncol.* 26:192-201 (1999). Among the drawbacks of whole cell vaccines is that it is labor-intensive and time consuming, especially if the cells are to be genetically modified. The success, or the lack of success, in the expansion of primary cultures for autologous vaccines can limit the courses of vaccinations and, further, an autologous vaccine needs to be specifically made for each patien. Simons et al., *Semin. Oncol.* 25:661-76 (1998). Another strategy for generating antigen-specific immunity is the *ex vivo* administration of specific antigen or peptides to antigen-presenting cells (APC). Again, this type of therapy is limited by the need to culture cells from each patient and success in the expansion of primary cultures for autologous vaccines can limit the number of courses of vaccination. Furthermore, the use of peptides to "load" APCs faces the obstacle of finding HLA-restricted peptides for all the different polymorphic HLA molecules. (Hwang et al., 1999).

[0007] The emergence of prostate cancer (CaP) as a major health issue and the absence of curative treatment for metastatic disease necessitate the development of new treatment modalities.

SUMMARY OF THE INVENTION

[0008] The present invention relates to the stimulation of specific cellular and humoral immune responses against prostate specific molecules in subjects vaccinated

with recombinant bacille Calmette-Guérin (rBCG) bacterial strains expressing prostate specific molecules.

[0009] Novel compositions and methods are provided for eliciting immune responses against prostate specific antigens. The immune responses are useful for treating prostate cancer.

[0010] Prostate-specific antigen (PSA) and prostate specific membrane antigen (PSMA) are two prostate-specfic TAA. PSA is expressed almost exclusively in normal, benign, and malignant prostate cells. Circulating PSA levels are frequently elevated with primary, locally recurrent or metastatic prostate cancer. Carr et al., *Cancer Res.* 55:2455-62 (1995). PSMA is predominantly found in the prostate and is upregulated in primary and metastatic prostate cancer. Moreover, PSMA has been observed in endothelial cells of capillary beds in certain tumors including those of the prostate. Therefore, PSMA may be targeted in tumor neovasculature as well as in carcinoma cells. Maraj et al., *Br. J. Urol.* 81:523-8 (1998).

[0011] In one aspect of the invention, rBCG strains are provided that express PSA. In another aspect of the invention, rBCG strains are provided that express PSMA or fragment thereof. Two particularly useful fragments are the amino-terminal 437 amino acids and the carboxy-terminal 446 amino acids.

[0012] The invention also provides methods of eliciting useful immune responses, particularly cell mediated responses, against PSA, PSMA or a fragment thereof in a mammal.

[0013] The invention further provides methods of treating prostate cancer, both organ limited and metastatic, in a mammal by eliciting an immune response against PSA, PSMA, fragments thereof, or a combination of the foregoing.

DESCRIPION OF THE FIGURES

[0014] Figure 1 depicts the quantitation of PSA in the BCG-PSA4 clone. Lane 1: negative control, lysate of BCG that expresses a control protein; Lane 2: lysate of BCG-PSA4; Lanes 3-8: two fold serial dilutions (200-6.25 ng) of human PSA (~32 kD, plus smaller breakdown products).

[0015] Figure 2 depicts expression of PSMA in BCG. Lane 1: positive control, lysate of influenza virus that has correct HA serotype (X-47E4940); Lane 2: negative control, lysate of influenza virus that has different HA serotype (PR8

E5201); Lane 3: lysate of BCG that expresses a control protein; Lanes 4 and 5: lysate of two clones of BCG2400 (clones 2400.2 and 2400.7, expressing full-length); Lane 6: lysate of BCG1300 (expressing amino-terminal 437 amino acids of PMSA); Lanes 7,8, and 9: lysates of three clones of BCG1500 (clones 1500.2, 1500.7, and 1500.9, expressing carboxyl-terminal 446 amino acids of PMSA).

[0016] Figure 3 depicts the DTH response in mice immunized with rBCG. Mice were challenged with either soluble PSA (Panel A) or PSMA (Panel B) and the response was quantified by increased footpad thickness (black bars: 24hours; white hatched bars: 48 hours) and histologic evaluation of infiltrates at 48 hours.

[0017] Figure 4 depicts the analysis of DTH reactions in mouse footpads 48 hours after elicitation by PSMA in a mouse vaccinated with rBCG-PSMA 12 weeks earlier. Mononuclear cell dermal infiltrates (arrowheads) are identifiable at 100X (Fig. 4A) and at 400X (Fig. 4B) magnification (hematoxylin and eosin).

[0018] Figure 5 depicts the antibody response to PSA in mice at 5 (hatched bars) and 10 (solid bars) weeks after immunization with PBS, PSA, rBCG-PSA or rBCG-PSMA1300. Sera not showing a response at the lowest dilution tested, 1:10, were assigned a titer of 10.

DETAILED DESCRIPTION OF THE INVENTION

[0019] The present invention is directed generally to vaccines and immunotherapeutics for treating prostate cancer. Specifically, the present invention is directed to presentation of prostate specific antigens to the host immune system to generate an immune response strong enough to have a therapeutic effect. The inventor has discovered that potent immune responses and successful therapy can be achieved by presentation of prostate specific proteins by an intracellular pathogen. That is, the vaccines and methods of the invention provide a means to sensitize the host immune system to that it will react with certain self antigens that are expressed in tumor tissue and neoplasms and cancers from tumor tissue.

[0020] Mycobacteria have adjuvant properties among the best currently known and stimulate a recipient's immune system to respond to other antigens with great effectiveness. A particularly valuable aspect of the vaccines produced with BCG is that cell-mediated immunity is elicited, which is especially useful in cases where cell-mediated immunity is considered to be critical for effective treatment, for

example in treatment of neoplastic diseases. Although humoral responses also result, immune protection from mycobacterial infection has been shown to depend on the development of host type-1 T-helper (Th1) cell mediated responses. rBCG can also be effective in stimulating cytotoxic T lymphocytes. To promote cell mediated responses, certain cytokines may also be used. For example, IL-2 and granulocyte-macrophage colony-stimulating factor amplify production of IFN-γ, which is characteristic of a Th1 response. Administration of IL-2 can result in stronger cellular responses to BCG and rBCG vaccines.

[0021] The experiments that follow demonstrate that immunization of mice with recombinant BCG expressing prostate-specific antigens induces readily detectable, specific, cell mediated delayed type hypersensitivity (DTH) responses to PSA and PSMA. For PSA and PSMA, the ability of rBCG to elicit a cell-mediated response is highly desirable. Notably, cellular, rather than humoral immune responses are responsible for the rejection of transplanted tumors or allogeneic tissue. Advantageously, mycobacteria also stimulates long-term memory or immunity, such that a single inoculation can be used to produce long-term sensitization to protein antigens.

[0022] Historically, BCG has been used as a vaccine for tuberculosis and has a very low incidence of adverse effects. Further, it can be used repeatedly in an individual (e.g., as the basis of vaccines that present different immunogens).

[0023] In one embodiment of the invention, recombinant BCG and vaccines therefrom contain a single prostate specific antigen, for example, PSA, PMSA, or fragments thereof. In another embodiment of the invention, different rBCG strains can be used to present multiple antigens.

[0024] Polypeptides can be expressed in BCG recombinants under the control of a mycobacterial stress responsive promoter - typically a hsp60 or hsp70 promoter. In the present case, prostate specific antigens are expressed under the control of the BCG hsp60 promoter, but other hsp promoters may be used.

[0025] Recently, vectors have become available that are capable of shuttling between *E. coli* and BCG. That and the discovery of high-efficiency heat-shock promoters that work in both types of bacteria has facilitated genetic manipulation of BCG strains. Generally, two types of vectors are available. Nonintegrating plasmids

have the advantage of higher copy number, whereas integrating vectors provide stable expression in the absence of continued antibiotic selection. See, Stover et al., *Nature* 351:456-60 (1997). Either type of vector may be used according to the invention.

[0026] Although slow growing, having a generation time of 20-24 hours, BCG can readily be cultured by methods well known in the art. Accordingly, rBCG is easily prepared for storage and administration. Moreover, BCG vaccines can be prepared and freeze dried for reconstitution at the time of administration.

[0027] Recombinant BCG of the present invention can be administered by known methods. Vaccines can be administered using one or more routes, including, but not limited to, subcutaneous, intramuscular, intranasal, intraperitoneal, intradermal, oral, or inhalation. rBCG of the present invention survive within the recipient expressing and secreting prostate specific antigens *in situ*. They can be administered alone to produce a desired response, such as an immune response, or can be administered in combination with other agents in order to enhance or modify the resulting response.

[0028] In the methods of the present invention, a therapeutically effective amount of a recombinant BCG strain is administered. The term "administering" as used herein means delivering the antibodies of the present invention to a mammal by any method that may achieve the result sought. The term "mammal" as used herein is intended to include, but is not limited to, humans, laboratory animals, domestic pets and farm animals. "Therapeutically effective amount" means an amount of antibody of the present invention that, when administered to a mammal, is effective in producing the desired therapeutic effect. Desired therapeutic effects include, for example, tumor regression, or maintenance of responsiveness to test antigens. Various methods are available for monitoring immune responses. For example, individuals primed in vivo with exogenous or endogenous antigen have lymphocytes in their blood that maintain an immunological memory for the priming antigen. Stimulated of whole blood with a test antigen followed by the quantitative measurement of IFN-gamma in plasma can be used to measure an individual's cellular immune response.

[0029] In summary, rBCG expressing prostate-specific molecules provides an effective immunotherapy for prostate cancer and avoids the drawbacks of other

treatment methadologies. BCG engenders a strong, long-lived immune response due to the ability to survive for several weeks in the host's macrophages, which can eliminate the need for numerous vaccine boosts. Live BCG is currently used as the vaccine for tuberculosis and its safety is already well established. This is beneficial due to the fact that live, antigen-expressing recombinant BCG appear to be critical for providing strong, specific, cell-mediated immunity; dead recombinant BCG and live non-recombinant BCG mixed with antigens are significantly less effective

[0030] The experiments demonstrate that recombinant BCG strains are capable of eliciting an immune response against PSA and PSMA. Delayed type hypersensitivity was induced against both PSA and PSMA. A delayed antibody response to PSA in animals vaccinated with BCG-PSA is observed compared to vaccination with human PSA. Clinically, vaccines comprising recombinant BCG strains are particularly useful for stimulating an immune response against prostate molecules that can eradicate metastatic prostate cancer cells. The experiments show that recombinant BCG expressing prostate-specific antigens induces readily detectable, specific, cell mediated immune responses to PSA and PSMA, which can be used to eradicate undetected metastatic prostate cancer after radical prostatectomy.

[0031] Throughout this application, various publications, patents, and patent applications have been referred to. The teachings and disclosures of these publications, patents, and patent applications in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which the present invention pertains.

[0032] It is to be understood and expected that variations in the principles of invention herein disclosed may be made by one skilled in the art and it is intended that such modifications are to be included within the scope of the present invention.

[0033] The examples which follow further illustrate the invention, but should not be construed to limit the scope in any way.

EXAMPLES

[0034] Gene Clones - A cDNA clone for PSA was provided by Dr. Tim Ratliff (Washington University, St. Louis, MO.). A cDNA for PSMA was a gift from Drs. Warren Heston and Polly Gregor (Memorial Sloan-Kettering Cancer Center, New York, NY). Sequences for the gene, mRNA, coding sequence, and complete protein are available from GenBank (PSA: Genbank Accession No. NM001648; PMSA: Genbank Accession No. AF007544).

[0035] Expression Vectors - pMM7 is a mycobacterial expression shuttle vector engineered to express and secrete foreign genes products in BCG. It was constructed by inserting the BCG alpha antigen signal sequence (AASS) between the heat shock protein (hsp) 60 promoter and the multicloning site of the pmv261 expression vector. O'Donnell et al., *Infect. Immun.* 62:2508-14 (1994). cDNAs of interest are inserted into the multicloning site of the vector and are constitutively expressed by the hsp 60 promoter. The AASS was inserted to allow for the possible secretion of protein products by the bacteria. Recombinant proteins produced by pMM7 are initially expressed as a fusion protein with the AASS, which is cleaved off during secretion. Recombinant BCG clones are selected in the presence of 50 μg per ml of kanamycin, the selectable marker on pMM7.

[0036] pMM7-HA is identical to pMM7 except for the insertion of an oligonucleotide tag (Flu+ GATCCAGCTTACCCATACGACGTCCCAGACTACGCTGCTACAG) coding for the influenza hemagglutinin (HA) sequence, YPYDVPDYA, between the AASS and the multi-cloning site.

[0037] Transformation of E.coli - The pMM7-PSA and pMM7-HA-PSMA plasmids were electroporated, separately, into XL1-Blue *E. coli*. Electroporation was performed using the BioRad Gene Pulser ® Apparatus at the following settings: 25uF, 2.5kV, 200 ohms. After electroporation, 500ul of warm SOC medium was added, and the cells were incubated for 30 minutes at 37^0C to allow for recovery and antibiotic resistance expression. The samples were plated separately onto L-broth agar plates (Life Technologies, Rockville, MD) with 50 ug/ml kanamycin (Sigma, St. Louis, MO) and incubated overnight at 37^0C. Kanamycin resistant colonies were picked and

grown in 4 ml of L-Broth with 50 μg/ml kanamycin overnight at 37^0C, with aeration. DNA was prepared using Qiagen miniprep extraction kits (Qiagen, Valencia CA) and clones were sequenced to ensure that the prostate specific cDNAs were in the correct reading frame.

[0038] Transformation of BCG - Constructs were electroporated, separately, into the Connaught strain of BCG, plated onto 7H10 Middlebrook agar (Difco, Detroit MI) containing 50 μg /ml kanamycin and incubated at 37^0C. Kanamycin resistant colonies were picked after approximately 6 weeks and grown in 7H9 Middlebrook broth, containing 50 μg /ml kanamycin, at 37^0C, with aeration (Difco, Detroit MI), until an OD_{600} of approximately 1.0. BCG were harvested by centrifugation and washed 2 times with PBS containing 0.02% Tween-80 and disrupted by sonication with a Branson Sonifier (output control 7, duty cycle 50%, 4 minutes) in RIPA buffer containing a protease inhibitor cocktail composed of: 0.01 mg/ml Aprotinin, E-64, Leupeptin, and Pepstatin A, and 0.50 mg/ml Pefabloc in DMSO.

[0039] Western Blot Analysis BCG lysates were diluted 1:1 with 2x electrophoresis loading buffer (Sambrook et al., Molecular: Cloning, A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, 1989), boiled and size fractionated by SDS-PAGE using a Novex minigel apparatus (Novex, San Diego CA). BCG-PSA lysates were fractionated on 12% pre-cast Novex Tris-glycine gels and BCG-PSMA lysates were separated on 10% Novex NuPage gels. Fractionated proteins were transferred to Immobilon-P membrane (Millipore, Bedford, MA) using a BioRad Trans-Blot® SD apparatus at 12V for 45 minutes using 20 percent transfer buffer (Sambrook et al.) The membrane was blocked with 5% blocking solution (PBS containing 2.5% dry milk and 2.5% BSA) for 1 hour on a rotating platform, at room temperature, and rinsed once with PBS. The blot was then incubated, overnight, on a rotating platform at 4°C, with a 1:100 dilution of a mouse anti-PSA monoclonal antibody, ER-PR8 (Dako, Carpinteria, CA) or a 1:1000 dilution of anti-HA tag (Covance Richmond, CA) accordingly. The blot was washed 3 times with PBS containing 0.05% Tween, and once with PBS (10 minutes each) and incubated with horseradish peroxidase (HRP)-labeled , rat anti-mouse IgG_1 antibody (1:1000 dilution; PharMingen, San Diego CA) for 45 minutes at room temperature, and washed as above. A 1:1 mixture of the ECL Western Blot Systems (Amersham

Pharmacia Biotech, Piscataway NJ) solutions was applied to the blot for 1 minute with vigorous shaking. Excess moisture was removed and X-ray film (Marsh Biomedical Products, Rochester, NY) was exposed to the blot in a dark room for 5 minutes then processed by a Kodak M35A X-OMAT Processor (Rochester, NY). Human PSA (Scripps Laboratories,San Diego, CA), influenza virus (gift from Dr. Edwin Kilborne, New York Medical College, Valhalla, NY) and HA tagged molecular weight markers (Roche Pharmaceuticals, Indianapolis IN) were used as standards and/or positive controls.

[0040] The densitometry used to calculate the protein concentration on autoradiographs was performed using an Alpha Imager ™ 2000, IS-1000 Digital Imaging System (Alpha Innotech Corp., San Leandro, CA).

[0041] Cloning and Expression of PSA and PSMA. - The entire coding region of PSA was cloned into the *Pst I/Hind* III restriction sites of pMM7. Following electroporation into BCG, two clones were identified that expressed PSA. Western blot analysis followed by densitometry, using known amounts of purified human PSA as standards, was used to quantitate PSA expression. The lysate from clone BCG-PSA4 contained approximately 24 ng of PSA per microgram of BCG protein (Fig. 1).

[0042] The entire coding region of PSMA was cloned into the *Pvu* II restriction enzyme site of pMM7-HA. Following electroporation into BCG, two clones were identified that expressed PSMA. As depicted in Fig. 2, PSMA is detected as an approximately 86kD band. As PSMA is a large protein (750 amino acids) it was anticipated that it may not be efficiently expressed in BCG. Therefore, two overlapping fragments of PSMA were individually cloned into pMM7-HA. One fragment contains the 5-prime 1314 nucleotides (encoding the amino-terminal 437 amino acids of PSMA; clone BCG-PSMA1300). The second fragment contains the 3-prime 1483 nucleotides of PSMA (encoding the 446 carboxyl-terminal amino acids of PSMA; clone BCG-PSMA1500). Both of these fragments are efficiently expressed in BCG (Fig. 2; BCG-PSMA1300: 0.66 ng PSMA/μg of BCG protein; BCG-PSMA1500: 0.47 ng PSMA/μg of BCG protein).

[0043] PSMA expression in lysates was quantitated using serial dilutions of a known 30kD HA tagged molecular weight marker on Western Blot analysis, followed by densitometry. The lysate of BCG-PSMA2400.2 contained approximately 112 pg

of PSMA/μg of BCG protein. Clone BCG-PSMA2400.7 has approximately 370 pg of PSMA/μg of BCG protein.

[0044] Immunization of mice - Six to ten week old (C57BL/6 x BALB/c) F1 (CBF1) mice were obtained from Jackson Laboratories (Bar Harbor, Maine). CBF1 mice were subcutaneously injected with one million colony-forming units of rBCG-PSA, rBCG-PSMA1300 (expressing the 5'-1314 nucleotides encoding the amino terminal 438 amino acids of PSMA), BCG (with vector only), 5 μg of PSA protein, 5 μg of PSMA protein or PBS (100 μl total volume).

[0045] Detection of DTH - Groups of 5 immunized and control mice were challenged 12 weeks after vaccination with 10 μg PSA or 5 μg PSMA in 10 μl of PBS into the footpad using 100 μl Hamilton syringe fitted with a 30 or 26 gauge needle. Footpad thickness was measured by a vernier caliper, prior to and 24 and 48 hours after challenge. The mice were then sacrificed and the hind paws were removed, fixed in formalin, embedded in paraffin, and coded hematoxylin and eosin sections were evaluated for mononuclear cell infiltrates. Sections were scored as 0 (no infiltrates) to 5 (extensive infiltrates and necrosis), and scores were averaged. Differences in footpad thickness and differences in mean infiltrate intensity analyzed statistically by one-way analysis of variance (ANOVA) with a Tukey-Kramer multiple-comparison post-test. Coded sections were also stained for non-specific esterase (NSE, Sigma Chemical Co., St. Louis, MO) according to the manufacturer's instructions for evaluation of macrophage infiltrates. Results are reported as per cent macrophages per hundred infiltrating mononuclear cells.

[0046] BCG-PSA and BCG-PMSA induced specific DTH as measured by increases in footpad thickness that were maximal at 24 h which could only be elicited by the homologous protein antigen. Thus, only mice immunized with BCG expressing PSA exhibited a significant increase in footpad thickness in response to challenge by the PSA protein (Fig. 3A). Similarly, only mice immunized with BCG expressing PMSA exhibited a significant increase in footpad thickness in response to challenge with PMSA protein (Fig. 3B). PSA protein elicited significantly more intense mononuclear cell infiltrates at 48 h in animals immunized with rBCG-PSA or with rBCG-PMSA than in animals immunized with PSA or PBS ($P < 0.01$, Fig. 3B). PMSA elicited significantly more mononuclear cell infiltrates at 48 h in animals

immunized with PSMA or with rBCG-PMSA than in animals immunized with BCG-PSA or PBS ($P < 0.01$, Fig. 3B). An example of the mononuclear cell infiltrates seen in these reactions is shown in Figure 4. Forty-five percent of the infiltrating cells were esterase-positive macrophages (data not shown). No neutrophil infiltrates were observed in any section. PMSA protein elicited some mononuclear infiltrates even in animals immunized with PBS (Fig. 3B). This may be due to the solubilization of PSMA in urea which would have contributed to the presence of inflammatory cells at the site of injection resulting in a microscopic response in the absence of measurable foot pad swelling. Nevertheless, our data suggest that immunization with rBCG that express prostate specific molecules can stimulate cellular immune responses against the recombinant proteins.

[0047] Detection of an Antibody Response - Individual samples of serum were collected from each mouse by retro-orbital bleeding prior to, and 5 and 10 weeks after immunization. Equal amounts of sample from animals in each group were pooled. ELISA was used to assay anti-PSA and PSMA antibodies. Ninety six-well Immunlon-2 plates were coated with 100 ng/well of PSA in 100 μl of coating buffer (0.1 M $NaHCO_3$ pH 9.6), and incubated overnight at 37^0C after which the excess liquid was decanted. One hundred micrograms of PSMA solubilized in 8M urea or PSA was coated onto wells and incubated overnight at 4^0C after which the excess liquid was decanted, the plate was rinsed once with PBS, and blocked with 200 μl of 5% BSA in PBS per well for 1 hour at room temperature. To measure antibody activity, samples (100 μl) diluted in 1% BSA-0.05% Tween 20-PBS were then added to wells and incubated for 1 hour at room temperature. Preimmune sera were diluted 1:20, post-immune sera were serially diluted 2-fold (1:20 to 1:2560). The plate was then washed 5 times with 0.05% Tween-20 in PBS and once with PBS, and 100 μl HRP-conjugated rabbit anti-mouse IgG (H+L) (Jackson ImmunoResearch Laboratories, West Grove, PA) diluted 1:1000 in 1% BSA-0.05% Tween-20- PBS was added. After a 1 hour incubation at room temperature, the plate was washed as above, and colorimetric analysis was performed using TMB Microwell Peroxidase Substrate System (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD). Antibody titers were defined as the reciprocal of the highest dilution at which the

values of absorbance at 450nm (A_{450}) were 2 standard deviations greater than those obtained with pre-immune sera diluted 1:20.

[0048] Mice immunized with rBCG-PSA generated a weak antibody response to PSA, which became detectable (titer = 320) at week 10. Mice immunized with human PSA had a high antibody titer (1250) at 5 weeks which decreased significantly (160) by week 10 (Fig. 5). Immunization with BCG containing only vector or with PBS did not stimulate an anti-PSA antibody response. Similar results were obtained in a second duplicate experiment (data not shown).

[0049] The ingredients and method steps should be understood as examples that are intended to be illustrative only. In particular, the invention is not intended to be limited to the methods, protocols, conditions and the like specifically recited herein, insofar as those skilled in the art would be able to substitute other conditions, methods, amounts, materials, etc. based on the present disclosure to arrive at compounds within the scope of this disclosure. While the present invention is described with respect to particular examples and preferred embodiments, the present invention is not limited to these examples and embodiments. In particular, the compounds of the present invention are not limited to the exemplary species recited herein.

What Is Claimed Is:

1. A method of eliciting an immune response against prostate specific antigen (PSA) in a mammal comprising administering an effective amount of a recombinant bacille Calmette-Guérin (rBCG) strain expressing PSA.

2. The method of Claim 1, wherein the immune response comprises a cell mediated response response.

3. The method of Claim 1, wherein the immune response comprises a humoral response.

4. A method of inducing an immune response against prostate specific membrane antigen (PSMA) comprising administering an effective amount of a recombinant bacille Calmette-Guérin (rBCG) strain expressing PSMA or a fragment thereof selected from the amino-terminal 437 amino acids and the carboxy-terminal 446 amino acids.

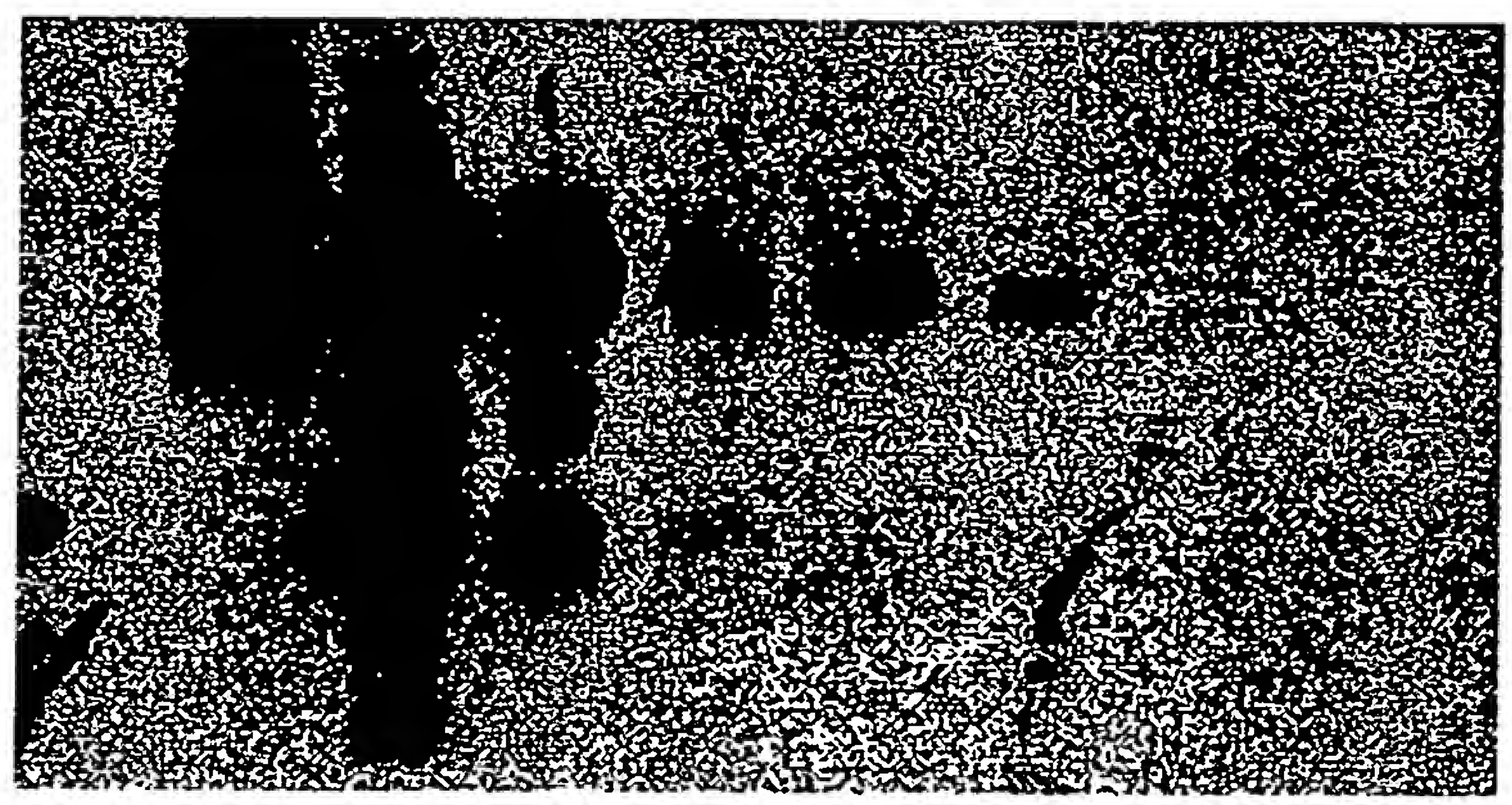
5. The method of Claim 4, wherein the immune response comprises a cell mediated response response.

6. A recombinant bacille Calmette-Guérin (rBCG) strain that comprises an expressible polynucleotide that encodes prostate specific antigen (PSA).

7. The recombinant bacille Calmette-Guérin strain of Claim 6, that expresses PSA.

8. A recombinant bacille Calmette-Guérin (rBCG) strain that comprises an expressible polynucleotide that encodes prostate specific membrane antigen PSMA or a fragment thereof selected from the amino-terminal 437 amino acids and the carboxy-terminal 446 amino acids.

9. The recombinant bacille Calmette-Guérin strain of Claim 8, that expresses PSMA or a fragment thereof selected from the amino-terminal 437 amino acids and the carboxy-terminal 446 amino acids.



Lane 1 2 3 4 5 6 7 8

Fig. 1

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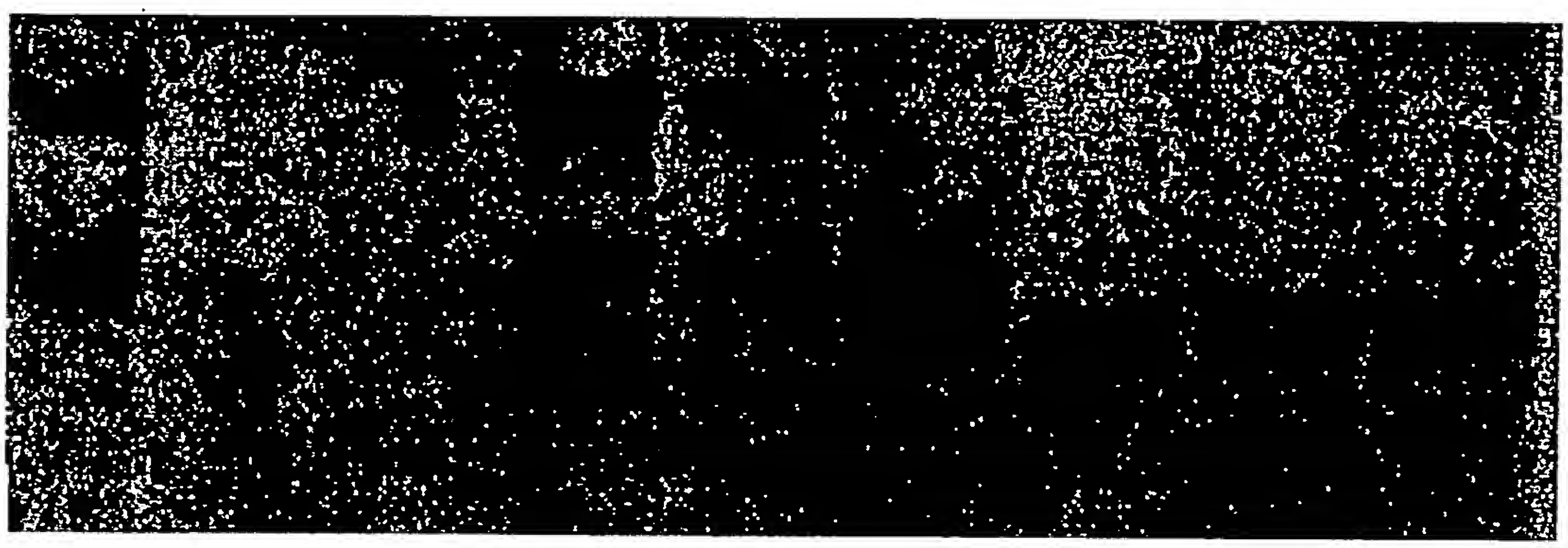
43kD



Lane 1 2 3 4 5 6 7 8 9

Fig. 2

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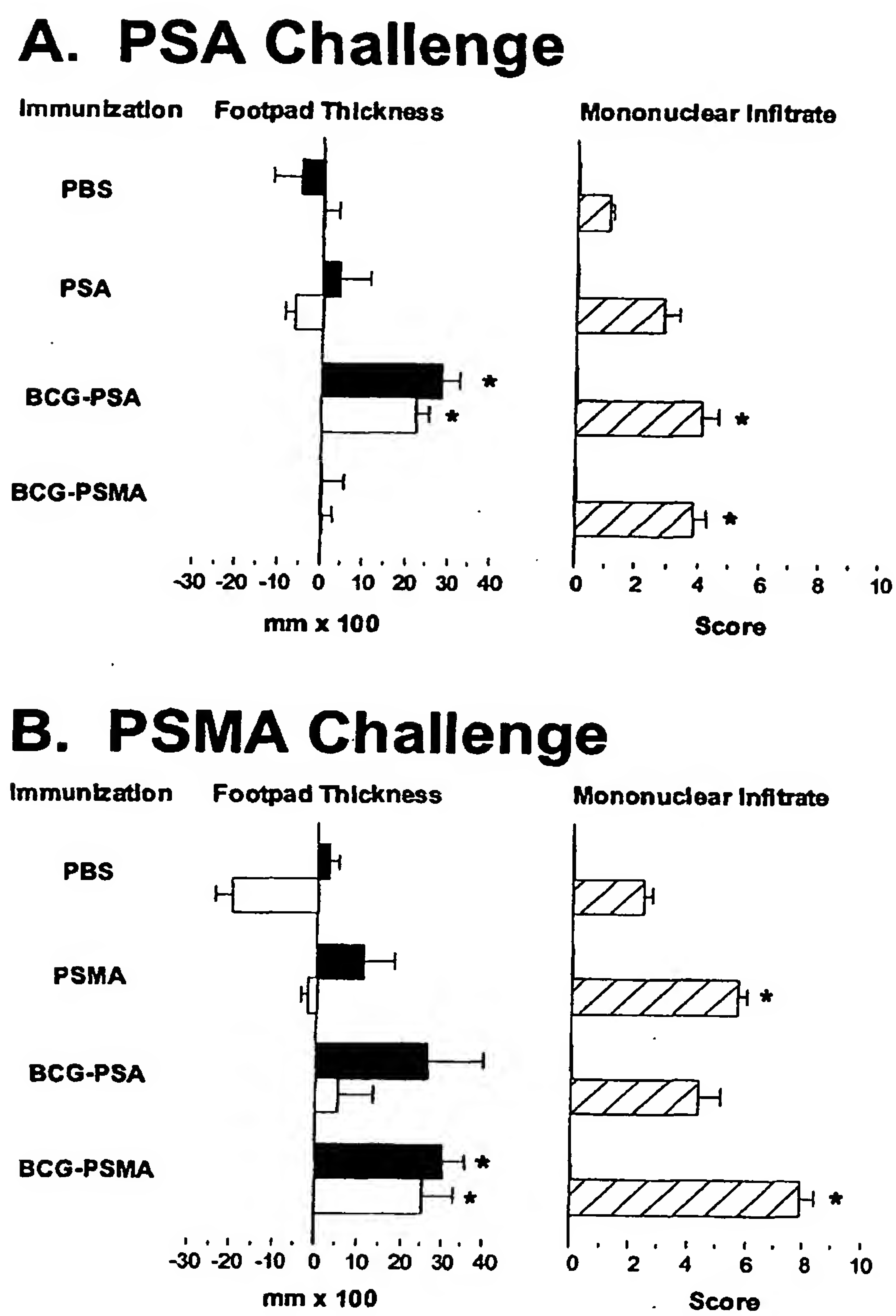

Fig. 3

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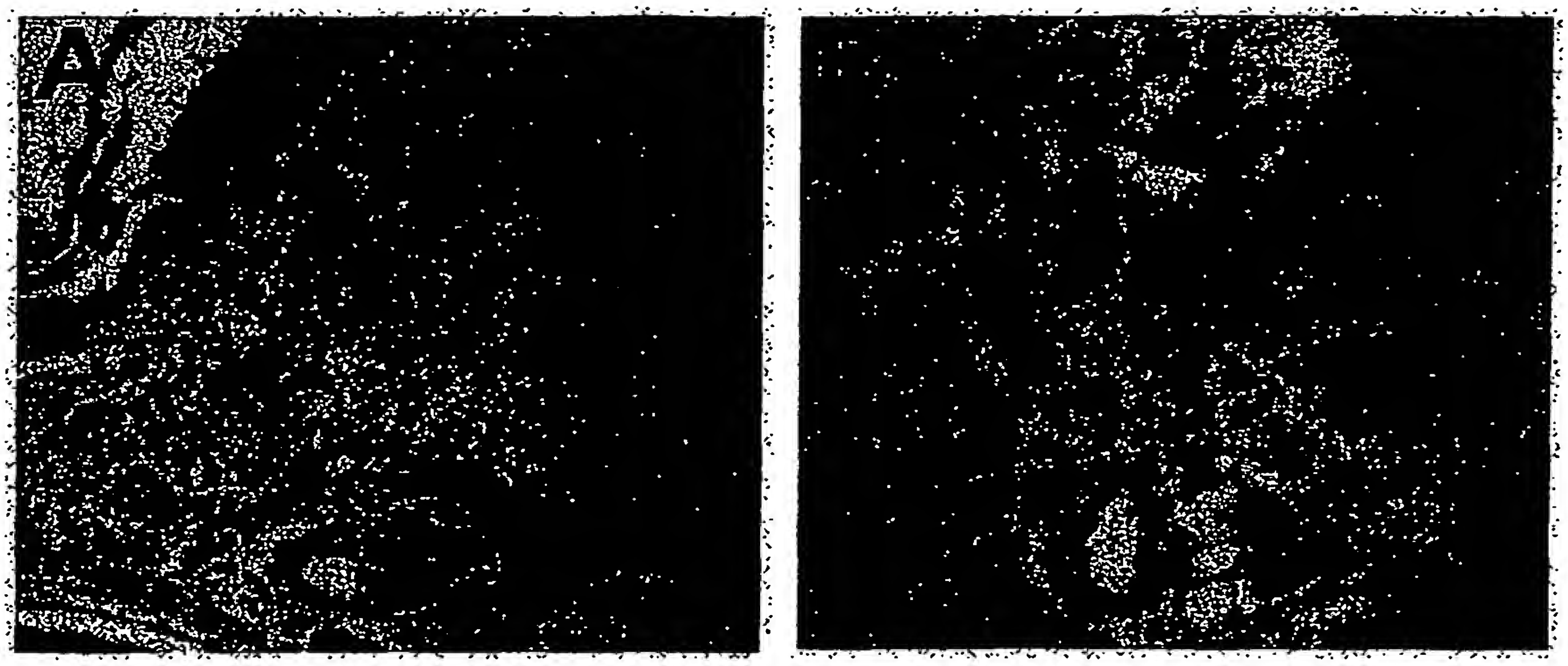

Fig. 4

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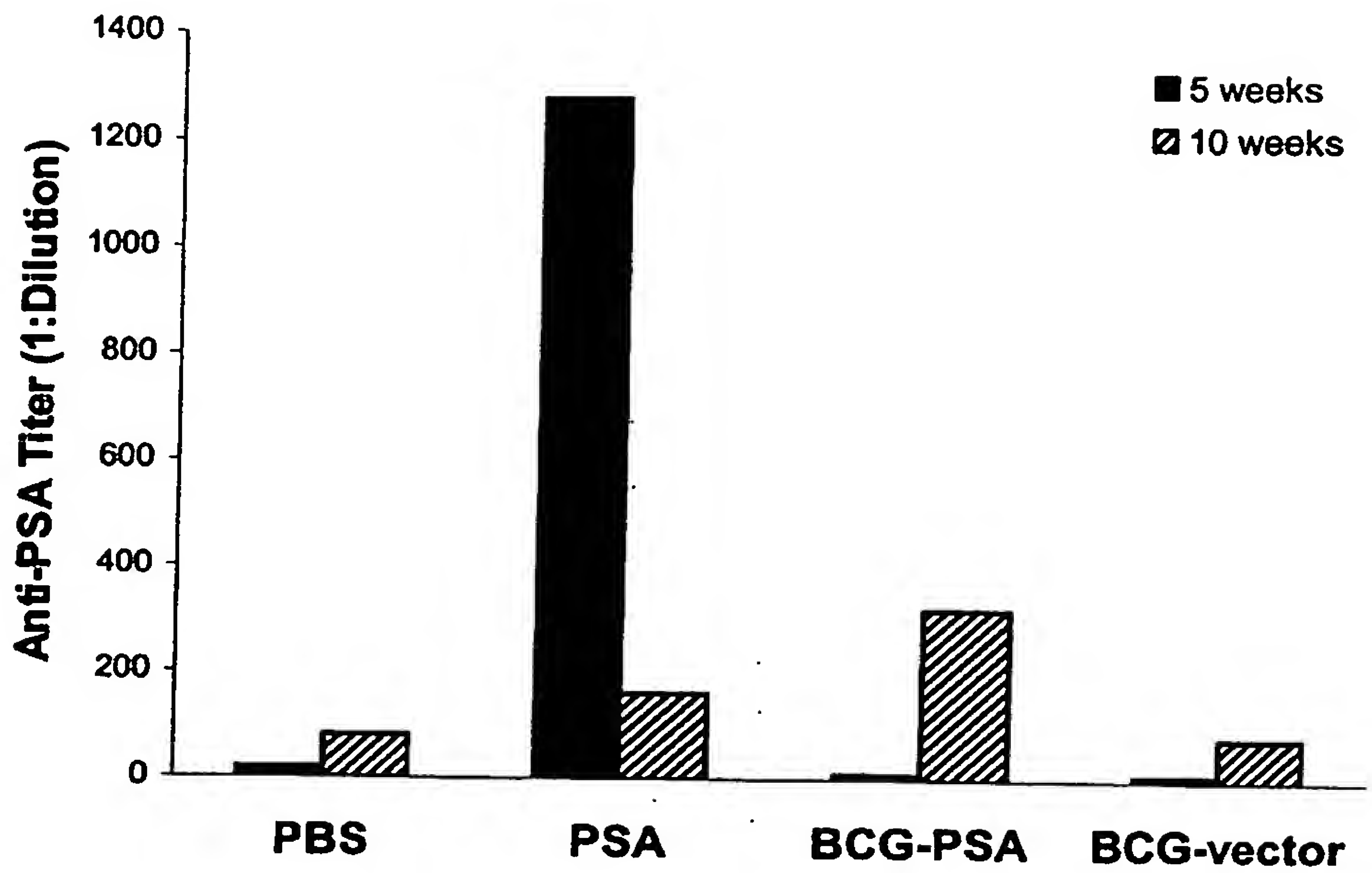

Fig. 5

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